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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/594,188	06/21/2007	Eugen Kolossov	2590.0040002/EJH/UWJ	7273

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EXAMINER	
CHEN, SHIN LIN	
ART UNIT	**PAPER NUMBER**
1632	
MAIL DATE	**DELIVERY MODE**
11/23/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/594,188	KOLOSSOV ET AL.	
	Examiner	Art Unit	
	Shin-Lin Chen	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *9-28-09 & 10-26-09*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13,17,19-32 and 45-70* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13,17,19-32 and 45-70* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *9-28-09*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10-26-09 has been entered.

Applicants' amendment filed 9-28-09 has been entered. Claims 1 and 45 have been amended. Claims 1-13, 17, 19-32 and 45-70 are pending and under consideration.

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 1-13, 17, 19-32 and 45-70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dang et al., June 26, 2003 (US 2003/0119107 A1, IDS) in view of Yan et al., February 2003 (US 20030027331 A1) and Kehat et al., 2001 (The Journal of Clinical Investigation, Vol. 108, No. 3, p. 407-414, IDS).

Claims 1-13, 17, 19-32 and 45-70 are directed to a method for producing embryoid bodies (EBs) from pluripotent cells, such as embryonic stem cells, comprising rocking a container containing a liquid single cell suspension culture of pluripotent cells until generation of cell aggregates and diluting the suspension and further rocking the container containing the suspension until formation of EBs, wherein said culture of pluripotent cells has a concentration of about $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml, wherein the cells could be cultured on embryonic mouse fibroblasts feeder cells before agitation of suspension culture, an embryoid body obtained from said method, and cardiomyocytes or tissue of cardiomyocytes obtained from said embryoid body. Claim 7 specifies the concentration of pluripotent cells is about $1x10^6$ to $5x10^6$ cells/ml. Claims 8-10 specify the suspension is cultured for about 6 hours, 16 to 20 hours and in T25 flasks, respectively. Claims 11 and 12 specify the dilution is 1:10 and the final concentration of EBs in the suspension culture is about 500/ml, respectively. Claims 51 and 52 specify the culture of pluripotent cells has a concentration of $0.1x10^6$ to $0.5x10^6$ cells/ml and the suspension is cultured for about 48 hours, respectively. Claim 53 specifies the EBs are diluted to about 100-2000 EBs/10ml. Claims 17 and 54 specify the cells are differentiated into cardipmyocytes. Claims 19-30 and 55-66 specify the cells are genetically engineered using a selectable marker, such as puromycin resistant gene, and/or a reporter gene, such as EGFP, under the control of a cell type-specific regulatory sequence. Claims 27 and 28 specify the marker

gene and reporter gene are contained on the same recombinant nucleic acid molecule and on the same cistron, respectively. Claims 69 and 70 specify the cell type-specific regulatory sequence is atrial- and/or ventricular-specific and is selected from promoters of alphaMHC or MLC2v, respectively. Claims 4 and 48 specify the cells are obtained from a murine ES cell line. Claims 5 and 49 specify the culture medium is IMDM with 20% FCS and 5% CO2. Claims 6 and 50 specify the culture condition comprises 37oC and 95% humidity.

Dang teaches a novel bioprocess where aggregation of ES cells and EBs are controlled, and the EBs can be generated with high efficiency and cultured in high cell density and well-mixed system (e.g. abstract). Some ES cell lines require aggregation of multiple ES cells to enable EB formation and the EB can be further differentiated into differentiated embryonic stem cells and/or tissue, such as cardiomyocytes or cardiac tissue (e.g. [0020]). The invention is not limited to ES cell but also include any spheroid forming cell type, such as adult pluripotent cells, embryonic germ cells, early primitive ectoderm-like cells and neuronal stem cells (e.g. [0016]). The ES cells are encapsulated to control cell aggregation such that each capsule gives rise to one embryoid body and each capsule contains a predetermined number of ES cells that are permitted to aggregate to from a single EB (e.g. [0019]). Dang teaches single cell liquid suspension cultures (scLSC) of ES cells (e.g. [0025], [0028]). Dang teaches a method for efficient formation of EBs and the culture of EBs in suspension at higher cell densities by controlling cell aggregation (e.g. [0051]). A method of culturing spheroid-forming cells, such as pluripotent cells in a bioreactor system where the culture conditions can be measured and controlled (e.g. [0052]). The bioreactor or culture system keeps the cells and/or spheroids in liquid suspension by stirring, but other methods or means, such as agitation of the system, can be used (e.g.

[0053]). Dang also teaches individual R1 ES cells expressing GFP protein under the control of a constitutively active promoter was encapsulated with a cyan labeled ES cell, and the two cell types survived and proliferated to form spheroid containing two sources of cells (chimeric spheroids). The technology of forming chimeric spheroids can be used to manipulate the differentiation of the pluripotent cells into specific types of tissue using cell specific signals (e.g. [0185]). Dang teaches introduction of a specific construct expressing a marker gene under the control of a cell or tissue specific promoter into ES cells such that the transfected ES cells can be expanded, differentiated and selected to generate the desired cell type of interest (e.g. [0076], [0089]). The cell lineage-specific promoter for cardiomyocytes is alpha-cardiac myosin heavy chain promoter and MLC2v (e.g. Table 9). The reporter gene could be Hygromycin resistance gene, the puromycin resistance gene or G418 resistance gene (e.g. [0094]). Dang further teaches culturing CCE murine embryonic stem cell in IMDM medium supplemented with 15% FBS at 37oC in humidified air with 5% CO2 (e.g. [0110]).

Dang does not specifically teach rocking single cell suspension of the pluripotent cells or the cell concentration of about $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml, 1:10 dilution, final concentration of EBs in the suspension culture, and culturing the suspension culture for about 6hr or 16-20hr, 20% FCS and 95% humidity. Dang also does not specifically teach the marker gene and reporter gene are contained on the same recombinant nucleic acid molecule or on the same cistron.

Yan discloses that homozygous stem (HS) cells are pluripotent cells (e.g. [0016]) and teaches trypsinizing the HS cells grow the HS cells in single cell suspension culture in 2 ml ES-LIF medium and culturing the cells as suspension cells in suspension culture at a density of 1-

$3x10^6$ cells to allow stem cells to form rounded spherical clusters known as embryoid bodies (EBs) for 4-6 days (e.g. [0293]). The isolated HS cells can be induced to differentiated into cardiomyocytes using technique known in the art such as Kehat (2001) (e.g. [0220]).

Kehat teaches culturing human ES cells in suspension and plated to form EBs, and spontaneously contracting areas appeared in 8.1% of EBs. Cells from said contracting area within EBs were stained positively with anti-cardiac myosin heavy chain, anti-alpha-actinin, anti-desmin, anti-cardiac troponin 1 and anti-ANP antibodies. The human ES cell-derived cardiomyocytes displayed structural and functional properties of early stage cardiomyocytes (e.g. abstract). ES cell clumps were grown in plastic petri dishes at a cell density of about $5x10^6$ cells in a 58 mm dish (e.g. p. 408, bridging left and right column).

It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to rock pluripotent cells in single cell suspension because Dang teaches single cell liquid suspension cultures (scLSC) of ES cells and a bioreactor or culture system that keeps the cells and/or spheroids in liquid suspension by stirring or other methods or means, such as agitation of the system. Rocking is a type of agitation (see specification [0043], "Agitating the suspension for about six hours on a rocking table", or the amendment, page 14, "the agitation (e.g. rocking)". Therefore, it would be obvious to one of ordinary skill in the art to rock the single cell suspension of the ES cells taught by Dang. It also would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to culture the ES cells at a concentration of $0.5x10^6$ to $5x10^6$ cells/ml or about $0.1x10^6$ to $1x10^6$ cells/ml, 1:10 dilution, with a certain final concentration of EBs in the suspension culture, and culturing the suspension culture for about 6hr or 16-20hr because Yan teaches growing pluripotent cells at a concentration

of 1-3x10^6 cells/2ml and Kehat teaches culturing ES cells at about 5x10^6 cells in a 58 mm dish. The concentration of 1-3x10^6 cells/2ml is within the range of 0.5x10^6 to 5x10^6 cells/ml or about 0.1x10^6 to 1x10^6 cells/ml. Determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill. Dang teaches a method of culturing spheroid-forming cells, such as pluripotent cells in a bioreactor system where the culture conditions can be measured and controlled. Culturing ES cells at these conditions or with certain final concentration of EBs would be obvious to one of ordinary skill in order to optimize the culture condition for the ES cells. Having the marker gene and reporter gene contained on the same recombinant nucleic acid molecule or on the same cistron would be obvious to one of ordinary skill because determining effective orientation of the marker gene and reporter gene is routine optimization of a result-effective variable and is obvious to one of ordinary skill. One of ordinary skill would orient the marker gene and reporter gene in a vector in order to optimize the expression of the marker gene and reporter gene at target cells.

It would have been prima facie obvious for one of ordinary skill in the art to culture ES cells in medium having 20% FCS and 95% humidity because Dang teach culturing ES cells in 15% FBS and humidified condition. FBS and FCS are the same and growing ES cells in 95% humidity would be obvious to one of ordinary skill in order to optimize the culture condition for the ES cells. Determining effective dose or conditions for culturing ES cells is routine optimization of a result-effective variable and is obvious to one of ordinary skill.

One having ordinary skill in the art at the time the invention was made would have been motivated to do so in order to form EBs from pluripotent cells and to manipulate the differentiation of the pluripotent cells into specific types of cell or tissue using cell specific

promoter as taught by Dang, to form EBs from HS cells as taught by Yan or to form EBs from human ES cells as taught by Kehat with reasonable expectation of success.

Applicants argue that Dang teaches improvement of stirred bioreactors and differentiation of cells in stirred cultures. Applicants cite Exhibit 1, Dang (2004, Exhibit 2) and Bauwens (2005, Exhibit 3) and argue that Dang put emphasis on stirred suspension cultures and stirred bioreactors. There are only to options for preventing aggregation of EBs: (1) static cultures and (2) stirred cultures as taught by Dang. In the agitation method (e.g. rocking) ES cells are much less exposed to shear stress as compared to spinner flasks, whereby the capacity of the cells to differentiate is not negatively influenced and more EBs can be obtained (amendment, p. 16-17). This is not found persuasive because of the reasons set forth above under 35 U.S.C. 103(a) rejection. Dang does not teach only the stirred suspension cultures. Dang teaches single cell liquid suspension cultures (scLSC) of ES cells and a bioreactor or culture system that keeps the cells and/or spheroids in liquid suspension by stirring or other methods or means, such as agitation of the system. Rocking is a type of agitation (see specification [0043], "Agitating the suspension for about six hours on a rocking table", or the amendment, page 14, "the agitation (e.g. rocking)". Therefore, it would be obvious to one of ordinary skill in the art to rock the single cell suspension of the ES cells taught by Dang. Since agitation puts less stress and less negative influence on ES cells, it would be obvious to one of ordinary skill in the art at the time of the invention that more EBs and differentiated cell of desire could be obtained by using agitation method.

Applicants cite declaration by Dr. Schwengberg and argue that the agitation method produced a higher yield of EB per ml and yield of differentiated cells per ES cell seeded. Dang does not teach or suggest that rocking a single cell suspension culture would produce a higher volume and density of EBs (amendment, p. 17-18). This is not found persuasive because of the reasons set forth above under 35 U.S.C. 103(a) rejection and the reasons set forth above. Since Dang teaches agitation method of single ES cell suspension culture, it would be inherent that rocking the ES cell culture would produce higher EBs. Further, the claims fail to recite any significant higher EB production as compared to stirred culture of ES cells.

Applicants argue that Yan and Kehat do not cure the deficiencies of Dang. Yan does not teach a method of generating EBs by rocking a single cell suspension of pluripotent cells. Kehat teaches using clumps of ES cell to generate EBs, not a single cell suspension. Kehat does not teach rocking a single cell suspension culture to produce a higher volume and density of EBs (amendment, p. 18-19). This is not found persuasive because of the reasons set forth above under 35 U.S.C. 103(a) rejection and the reasons set forth above.

Applicants argue that none of the cited references teach a method of producing EBs from pluripotent cells by rocking a single cell suspension with a concentration of $0.5x10^6$ to $5x10^6$ cells/ml or $0.1x10^6$ to $1x10^6$ cells/ml (amendment, p. 19). This is not found persuasive because of the reasons set forth above under 35 U.S.C. 103(a) rejection and the reasons set forth above.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shin-Lin Chen whose telephone number is (571) 272-0726. The examiner can normally be reached on Monday to Friday from 9:30 am to 6 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272-4517. The fax phone number for this group is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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